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Journal of Magnetism and Magnetic Materials 278 (2004) 117–121

Journal of magnetism and magnetic materials

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Comparative magnetic measurements of migratory ant and its only termite prey

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Received 9 July 2003; received in revised form 14 October 2003

Abstract

Termites and ants are social insects living organized in nests in castes. Behavioral studies with the migratory ant *Pachycondyla marginata* have shown that it conducts well-organized predatory raids toward nests of its only prey, the termite *Neocapritermes opacus*. The magnetic materials in these two insects were studied using a SQUID magnetometer for two orientations. The J_r/J_s and J_r/χ_0, ratios were calculated from the two insects hysteresis curves. These ratios are in the range of magnetite pseudo-single or multi-domain particle values. The magnetic material are distinguishable by H_c values (30 Oe for ants and 100 Oe for termites) and by the magnetization magnitude, which is about two magnitude orders higher in the termite than in migratory ant. The *Pachycondyla marginata* SQUID results show an anisotropy in the magnetic material arrangement while for *Neocapritermes opacus* termite it is revealed by FMR spectra.

PACS: 87.18.−h; 75.60.Ej; 76.60.Es

Keywords: Social insect; Termite; Ant; Hysteresis; Magnetite

Magnetic orientation and sensitivity in higher animals have been intensively studied [1,2]. Complex mechanisms are required for the detection of the geomagnetic field and magnetic signal transduction to be used in migration, foraging and other orientation processes. Despite the large number of papers on behavior, effects of magnetic field and magnetic material detection in a variety of animals, it is still unknown how animals detect the geomagnetic field. The most important hypotheses invokes the existence of magnetic nanoparticles, associated to some specialized cells. The effect of the interaction between the nanoparticles and the geomagnetic field can change cellular structures, transmitting the information to the nervous system. To confirm this hypothesis, the presence of magnetic nanoparticles has to be proved in some part of animals with sensitivity to the geomagnetic field [3,4]. Biomineralized magnetite seems to be a good candidate for the hypothesis of magnetic particle receptors [5,6].

Social insects present a complex behaviour when they are foraging and/or migrating [1]. Magnetoreception [7], that is poorly understood, is one of

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0304-8853/$ - see front matter
doi:10.1016/j.jmmm.2003.12.327

the mechanisms of orientation used by these insects, besides skylight polarization [8], chemical pheromones, landmarks, etc. Concerning magnetic orientation, the honeybee *Apis mellifera* has been the most studied insect, and magnetic nanoparticles were found in their abdomen [9]. The influence of geomagnetic field on the behavior of ants has been reported for the species *Solenopsis invicta* [10] and *Formica rufa* [11]. Investigations of abdomen tissues of *Solenopsis invicta* confirm the presence of ferrous iron [12] and ferromagnetic magnetic resonance (FMR) results suggest the presence of magnetite in this ant [13]. The migratory ant *Pachycondyla marginata* (*P.m.*) has very peculiar habits: living termites *Neocapritermes opacus* (*N.o.*) as a special food diet and migration in a preferential direction [14]. Electron microscopy results showed magnetite/maghemite particles in head, thorax and abdomen of these ants [15]. FMR temperature dependence analysis of this ant smashed abdomens [16] support the model picture of isolated magnetite nanostructures of about 13 nm in diameter and of cluster structures containing in average three single units. Magnetic and electron microscopy studies have identified 10 nm magnetite particles size in only two species of termites, *Amitermes meridionalis* (*A.m.*) and *Nasutitermes exitiosus* (*N.e.*) [17].

The prey–predator relation between *N.o.* termite and the *P.m.* oriented migratory ant and the presence of magnetic material in other termite species stimulated the search of this material despite the lack of magnetic effects studies. This paper compares oriented *N.o.* termite and migratory ant *P.m.* room temperature hysteresis loops and FMR spectra. Their magnetic parameters are compared with those of magnetite.

N.o. and P.m. workers were collected in the region of Campinas, São Paulo, Brazil. The insects were extensively washed with ethanol 80%. Ants were kept in this solution until used and termites were immediately dried after washing. Ants were dried for 1 h at 50°C just before measured and termites were kept dry in the freezer until transferred to the SQUID/EPR sample holder with vacuum grease. Room temperature hysteresis loops were obtained using a squid magnetometer.

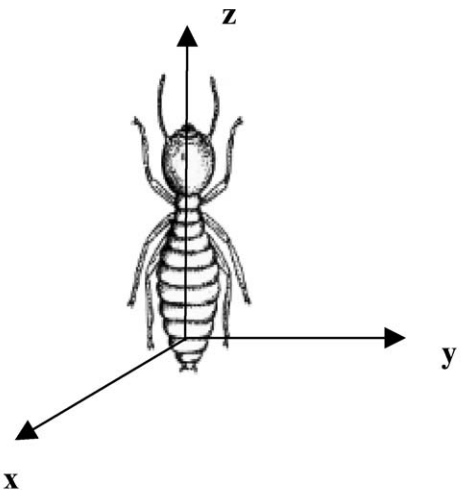

Fig. 1. Insects axis scheme.

(MPMS-XL Quantum Design) with samples oriented with the magnetometer magnetic field parallel to the body long axis (called z-axis) and to the perpendicular one (called x-axis) as indicated in Fig. 1. X-band termite FMR spectra (Bruker ESP 300E) with 4 mW microwave power and 2 Oe, field modulation amplitude, were obtained at RT in the same orientation above and with the magnetic field parallel to the third axis (called y-axis).

Fig. 2 presents the hysteresis loops normalized to one *P.m.* ant and one *N.o.* termite specimen, in the parallel orientation (magnetic field in the z body axis direction), both after subtracting the linear contributions in the high field ranges. Diamagnetism is dominant in ant while paramagnetism in termite curves. There is no report on magnetic properties of iron containing proteins, ferritin and haemosiderin in insects, particularly, in social insects although they are widely studied in mammals. Human liver ferritin magnetization is negligible at temperatures higher than 60 K [18]. The antiferromagnetic ordering temperature of horse spleen ferritin was reported to be approximately 240 K [19,20] and similarly for haemosiderin [19], while very low temperatures (20 K and approximately 3 K) were determined by Mossbauer spectroscopy for two bacterioferritin [21]. It is then assumed that at room temperature insect iron containing proteins contribute only with a

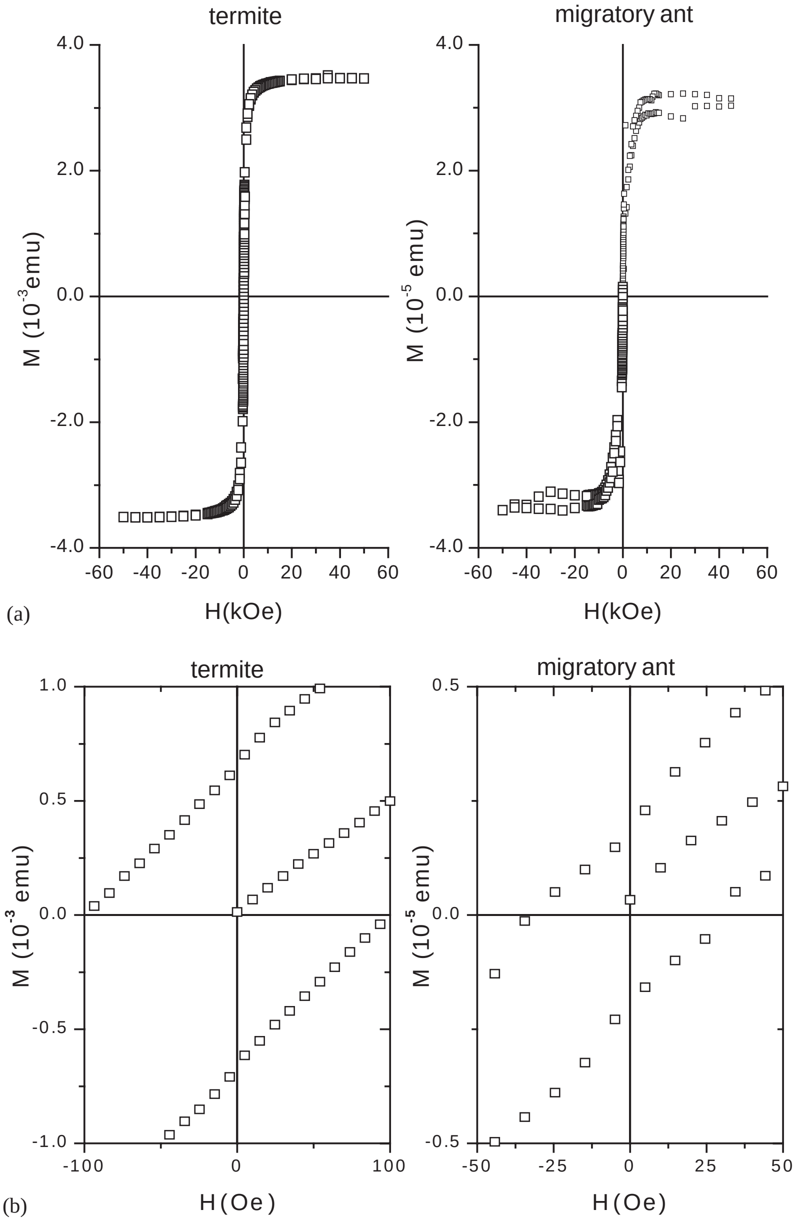

Fig. 2. (a) Room temperature hysteresis curves of one *P.m.* ant and one *N.o.* termite specimen with the magnetic field parallel to the body *z*-axis. (b) Details of (a) in the region of H_c and J_r.

paramagnetic phase. This contribution is not observed in ant loop because a diamagnetic one dominates it.

From those loops the coercive field (H_c), saturation magnetization (J_s), remanence magnetization (J_r) and initial susceptibility (χ_0) were obtained. Table 1 presents mean values of these parameters obtained in different experiments for both orientations. The ratios J_r/J_s, J_r/χ_0 are also given together with those characteristics of magnetite. These ratios and H_c values are clearly distinguishable in the two migratory ant

Table 1
Magnetic parameters from room temperature hysteresis loops of migratory ant, its termite prey (values obtained by subtracting the paramagnetic/diamagnetic contribution) and magnetite

	P.m. Ant	*N.o.* Termite	Magnetite	
	$H\|z$-axis/$H\|x$-axis	$H\|z$-axis/$H\|x$-axis	MD	SD
Length (mm)	10.7 ± 0.2	4.3 ± 0.4		
Diameter (mm)	2.2 ± 0.05	1.7 ± 0.06		
Mass (mg)	5.7 ± 0.5	1.0 ± 0.1	—	—
J_R (emu)	$(2.0\pm0.5/2.0\pm0.5)\times10^{-6}$	$(6.6\pm0.4/6.4\pm0.4)10^{-4}$	—	—
J_S (emu)	$(3.3\pm0.1/1.5\pm0.2)\times10^{-5}$	$(3.5\pm0.05/3.35\pm0.05)10^{-3}$	—	—
χ_o (emu/Oe)	$(4.5\pm0.5/2.9\pm0.1)\times10^{-8}$	$(5.1\pm0.07/4.1\pm0.05)\ 10^{-6}$	—	—
H_c (Oe)	$30\pm5/59\pm6$	$100\pm1/111\pm4$	—	—
J_R/J_S	$0.06\pm0.02/0.14\pm.05$	$0.19\pm0.01/0.19\pm0.02$	0.01–0.3[a]	0.3–0.5[a]
J_R/χ_o (Oe)	$460\pm160/70\pm20$	$135\pm25/156\pm12$	20–700 Oe[a]	20–700 Oe[a]

[a] Values from Ref. [2, p. 37].

orientations; the two ratios are higher when the field is parallel to the body x-axis while H_c is higher when the field is parallel to the body z-axis, evidencing an anisotropy in the ant magnetic properties. The termite magnetic parameters are the same within the experimental errors, in both directions except for H_c that is 10% higher in the x-axis orientation.

Based on magnetite particles values [22] J_r/J_s, J_r/χ_0 the insects' magnetic parameters are within the expected multi-domain (MD) region. Nevertheless, the termite J_r/J_s and H_c values follow the pure pseudo-single domain (PSD) or MD + PSD mixture particles [23]. These same parameters compared to those in Ref. [24] indicate termite grain size particles in the range from 100 to 220 nm and for migratory ant sizes larger than 220 nm, but different magnetic material cannot be discarded.

The analyses above were based on magnetite, as it is the more common magnetic material in animals. TEM of ant purified magnetic material [15] however, presented also another magnetic iron oxide, maghemite, a good candidate for magnetoreceptor, independent of magnetite degradation, probably produced by chemical extraction, and grain sizes that fit the SPM and SD region. Magnetic measurements resulted *N.o.* and *P.m.* grains in the region MD or MD + PSD, which indicate that some of these particles interact in clusters, similarly as observed in *A.m.* and *N.e.* termites [17]. For comparison, IRM magnetization of these two termite species were obtained, $0.2–1.4\times10^{-5}$ and 0.13×10^{-6} emu, respectively, values much lower than that of *N.o.* (see Table 1). The *N.o.* magnetization values are about two orders higher than those of the *P.m.* ant. *N.o.* termites present unexpected magnetic material contents as compared to other termites and to the *P.m.* ants, which worker body is about five times of the termite worker in volume and mass (Table 1).

Fig. 3 presents *N.o.* FMR spectra with the magnetic field parallel to the three-axis orientation and the *P.m* spectrum, to y-axis orientation. The *N.o.* spectra second integration are two orders higher than of this ant. FMR results confirm that *N.o.* contains much more magnetic material than their predator. The termite spectra with the magnetic field in the y-axis direction is characterized by a broad asymmetric line at $g = 2.15$ while in the z- and x-axis directions the line is resolved with a shoulder at $g = 2.67$ and 5.66, respectively (Fig. 3). Although the anisotropy is not so evident from the *N.o.* magnetization measurements, the FMR spectra in the third direction (y-axis) clearly show an anisotropy in the magnetic material arrangement The superimposed lines in the y-axis direction suggest a symmetry axis.

Conclusively, the anisotropy of the magnetic material studied in both insects reveals that it can play a sensory role, even if it is ingested or metabolic product. Moreover, the differences in organization, particle concentration and aggregation as well as interaction among them turn these

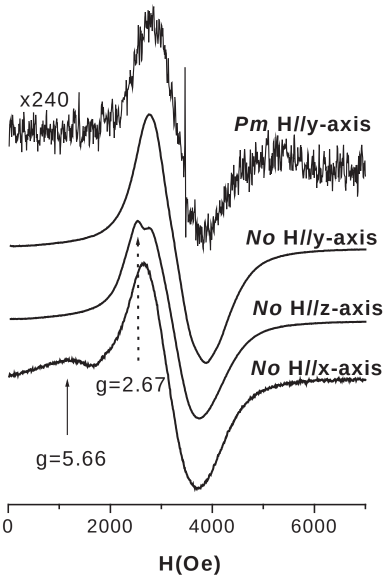

Fig. 3. RT termite FMR spectra with the applied magnetic field parallel to each axis indicated in the Fig. 1 scheme and to *y*-axis for migratory ant.

magnetic materials an interesting subject of study. This material in *P.m.* ants could be related to the observed oriented migration [25]. *N.o.* behavioral studies and migratory habits are not considered, as far we know, nevertheless magnetic effects in *Trinervitermes geminatus* termite foraging were reported [26]. Magnetic effects on *N.o.* termite behavior are difficult because of their poorly known habits, so that magnetic sensory function suggested was not observed yet. Could it arise as a survival strategy associated to the prey–predator system? This paper stimulates further ecological and evolutionary studies in this system.

We are grateful to Dr. Paulo Sérgio de Oliveira for collecting the insects and Dr. Henrique Lins de Barros for helpful comments on this paper. D.M.S.E and E.W thank FAPERJ and CNPq for financial support.

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